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COELOMIC FLUID VOLUME REGULATION AND ISOSMOTIC INTRACELLULAR REGULATION BY *LUIDIA CLATHRATA* (ECHINODERMATA: ASTEROIDEA) IN RESPONSE TO HYPOSMOTIC STRESS

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Despite an inherent inability to control the osmotic pressure of their body fluids, many echinoderms are able to tolerate hyposmotic environments (Binyon, 1966, 1972a). Among asteroids, euryhalinity has been demonstrated and studied in detail only in the genus *Asterias* (Meyer, 1935; Maloeuf, 1937; Smith, 1940; Loosanoff, 1945; Bock and Schlieper, 1953; Kowalski, 1955; Schlieper, 1957, 1958; Binyon, 1961, 1962, 1972b; Jeuniaux, Bricteaux-Grégoire and Florkin, 1962). Reproductively active populations of *Asterias rubens* are found in the Baltic Sea in salinities ranging from 15–17‰ (Kowalski, 1955). Reproductively active populations of the evolutionary primitive platyasterid asteroid, *Luidia clathrata* (Say), similarly are found in Tampa Bay, Florida (Lawrence, 1973). Salinities in the bay range from 18–27‰ as a result of seasonal changes in rainfall. As a consequence of the low and fluctuating salinity levels, this asteroid must be able to tolerate or compensate for large variations in the osmotic pressure of its coelomic fluid.

Preliminary observations indicated that specimens of *Luidia* were able to survive salinities as low as 14‰. Sudden exposure to this low salinity resulted in the cessation of podia activity and overall swelling of the body as has been reported for *Asterias rubens* subjected to hyposmotic stress (Binyon, 1961, 1972b; Jeuniaux *et al.*, 1962). Recovery of activity by these hyposmotically stressed specimens of *Luidia* indicated that compensatory processes were occurring. Florkin and Schoffeniels (1969) have suggested that isosmotic intracellular regulation occurs when extracellular anisosmotic regulation is not present in euryhaline invertebrates. Changes in the intracellular levels of free amino acids associated with isosmotic intracellular regulation have been reported for the echinoid, *Strongylocentrotus drobachiensis* (Lange, 1964) and the asteroid, *A. rubens* (Jeuniaux *et al.*, 1962; Binyon, 1972b).

The purpose of the present study was to ascertain if coelomic fluid volume regulation and intracellular regulation occur in hyposmotically stressed specimens of *Luidia clathrata*, and to associate their time courses with that of the recovery of activity of the intact animal. In addition, the time course of the rate of ammonia excretion of hyposmotically stressed animals was measured to observe if it could be correlated with that of expected changes in the levels of ninhydrin positive substances in the body tissues.

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Materials and Methods

Experimental animals

Animals were collected in Tampa Bay, Florida in April, 1973. Ambient temperature was 24° C and ambient salinity was 27‰. The animals were maintained without food in the laboratory in well-aerated, continuously filtered aquaria. Thirty animals each were placed in hyposmotic artificial sea water (Seven Seas Mix; 16‰) and ambient sea water (27‰). Five animals from each group were removed after 1, 2, 4, 6, 8 and 10 days for analysis.

Whole animal analyses

The volumes of the whole animals were determined by measuring the amount of sea water they displaced. By marking the epidermis with nile blue, it was possible to determine the volume of each individual animal at the beginning of the experiment and again when each was used for analysis. From this, the volumes of the animals through the experimental period were calculated as the per cent of its original volume.

The activities of the whole animals were measured by utilizing the activity coefficient (1000/righting time in seconds) as used by Percy (1971) for echinoids. The righting of inverted animals is a basic reflex of echinoderms which involves considerable neuromuscular coordination (Reese, 1966) and should reveal the functional well-being of the animal. Following Percy, the mean activity coefficient ± a standard deviation was used when all individuals in an experimental group righted within a set period of time (10 minutes), and the median activity coefficient was used when not all individuals righted within that period.

Tissue analyses

After the measurements were made on the intact animals, the podia and pyloric caeca were removed from each animal. The podia were scraped from the ambulacral grooves with a spatula and blotted with filter paper to absorb the ambulacral fluid. The pyloric caeca were dissected from the body cavity. Portions of each tissue were weighed, dried in a vacuum desiccator over concentrated sulfuric acid, and reweighed to obtain the per cent hydration.

Ninhydrin positive substances (NPS) were measured by the method of Cocking and Yemm (1954) on tissue extracts prepared with 5% TCA. Alanine was used as the standard. Protein was measured by the method of Lowry, Rosebrough, Farr and Randall (1951) on extracts prepared with 1 N NaOH. Bovine serum albumin was used as the standard. The concentrations of NPS were calculated in terms of g tissue water.

Ammonia excretion

Rates of ammonia excretion were ascertained on animals different than those used above. Five animals each were placed in hyposomotic (16‰) and ambient (27‰) sea water. After 2, 6, 12, 26, and 52 hours, the animals were placed in individual bowls which contained 100 ml of sea water of the same concentration as that from which they had been taken. At the end of 2 hours, water samples

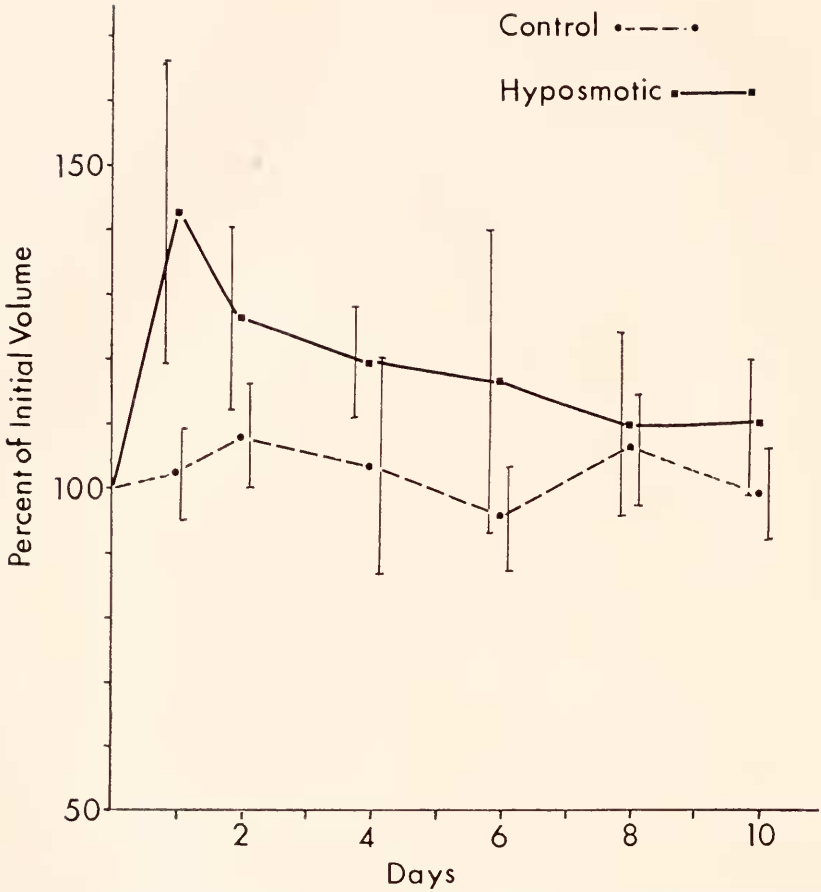

FIGURE 1. Changes with time of the per cents of initial whole animal volume of *Luidia clathrata* maintained at control (ambient) salinity (27‰) and at hyposmotic salinity (16‰). Points represent means (n = 5) ; vertical lines represent ± 1 s.d.

were removed from the bowls and frozen immediately. The animals were returned to their aquarium until the next incubation period. This procedure avoided the buildup of organic material in the water surrounding the animals which might have occurred otherwise. Ammonia was determined by the microdiffusion method of Conway (1962). Ammonium sulfate was used as the standard. The rate of ammonia excretion was calculated as μg NH_3-N/g dry weight of the animal/hour.

RESULTS

Whole animal analyses

Introduction of *Luidia* into the hyposmotic medium resulted in immediate cessation of movement. Podia were insensitive to mechanical stimuli. Activity of the animals resumed within 48 hours. No deaths occurred in either the hyposmotically

stressed animals or the controls in the experiment. The volumes of hyposmotically stressed animals at day 1 were greater than initially (Fig. 1). Subsequently, the per cents of initial volumes declined with time. The per cents of initial volumes of the animals at ambient salinity were close to 100% throughout the experimental period, indicating no significant volume changes. The per cents of the initial volume values of the two groups were significantly different at the 95% level at day 1 but not at subsequent times.

The hyposmotically stressed animals did not right themselves within 10 minutes at the end of day 1 (Fig. 2). The activity coefficients of the hyposmotically stressed animals increased subsequently and became similar to initial levels, indicating recovery of functional well-being.

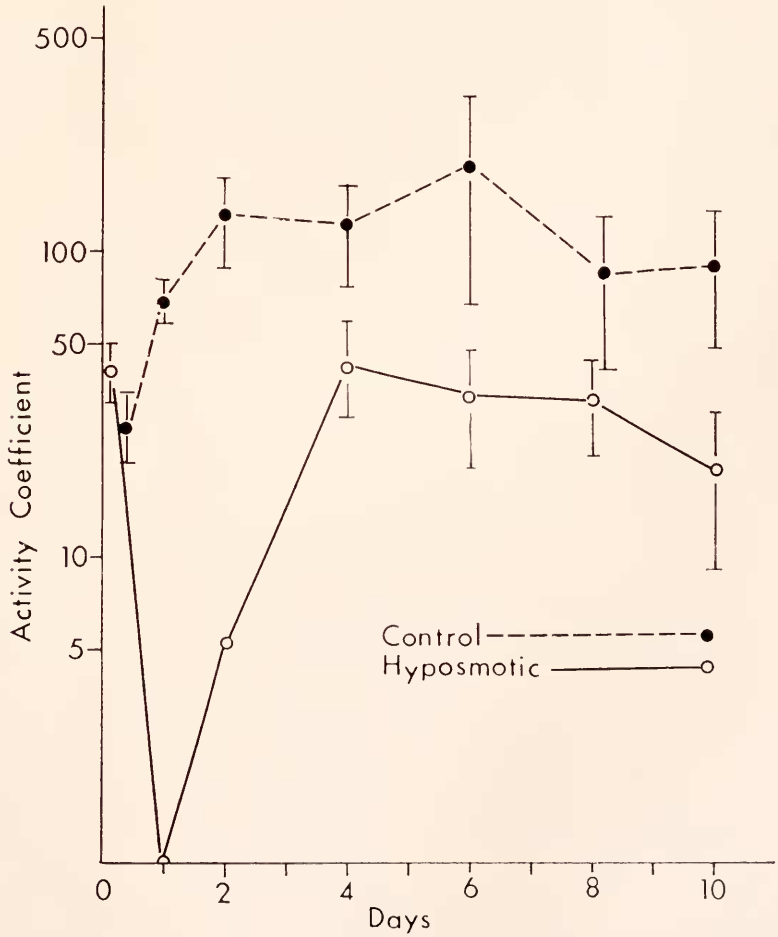

FIGURE 2. Changes with time of the activity coefficients of *Luidia clathrata* maintained at control (ambient) salinity (27‰) and at hyposmotic salinity (16‰). Points represent means (n = 5) except for hyposmotically stressed animals at day 2; vertical lines represent ± 1 s.d. The exception is a median value as one individual failed to right.

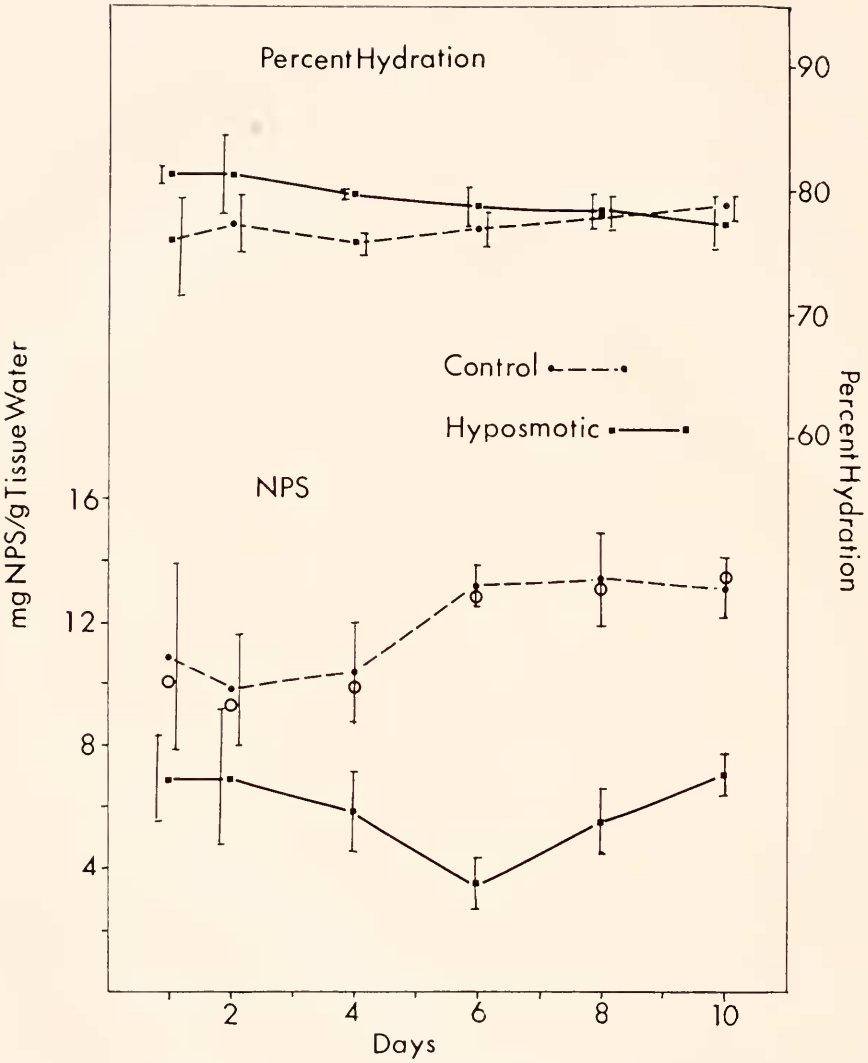

FIGURE 3. Changes with time in the per cents hydration and levels of ninhydrin positive substances (NPS) in the podial tissues of *Luidia clathrata* maintained at control (ambient) (27‰) and at hyposmotic salinity (16‰). Solid symbols represent means ($n = 5$); vertical lines represent ± 1 s.d. Open symbols represent expected levels of NPS calculated on the basis of changes in tissue hydration.

Tissue analyses

At the end of day 1, the per cents hydration of the podia and the pyloric caeca were significantly higher at the 95% level than those in the tissues of control animals. The per cent hydration of the podia of hyposmotically stressed animals decreased after the first day, eventually equalling the levels in the tissues of control

animals (Fig. 3). The per cent hydration of the pyloric caeca of hyposmotically stressed animals decreased after the first day, but remained higher than the levels in the tissues of the control animals (Fig. 4).

The changes in the per cent hydration of both tissues were paralleled by changes in the levels of NPS. The levels of NPS in the podia and the pyloric caeca of hyposmotically stressed animals were lower than those in the tissues of control

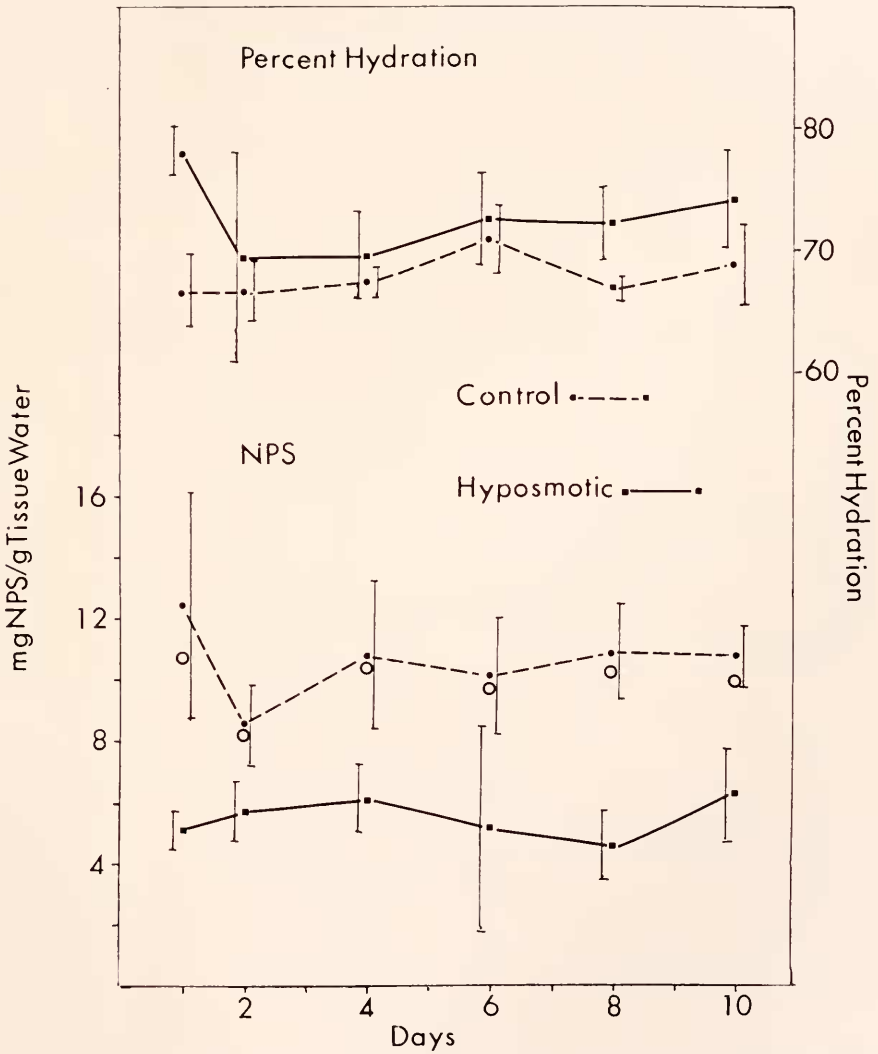

FIGURE 4. Changes with time in the per cents hydration and levels of ninhydrin positive substances (NPS) in the pyloric caeca of *Luidia clathrata* maintained at control (ambient) salinity (27‰) and at hyposmotic salinity (16‰). Solid symbols represent means ($n = 5$); vertical lines represent ± 1 s.d. Open symbols represent expected levels of NPS calculated on the basis of changes in tissue hydration.

animals by the end of day 1, and remained lower throughout the experimental period. Except for the level in the podia on day 2, the differences in the levels of NPS in both tissues of the stressed animals were significantly different at the 95% level from the levels in the tissues of control animals. Expected changes in NPS levels calculated on the basis of changes in tissue hydration constituted only a small fraction of the decrease actually found in the hyposmotically stressed specimens of *Luidia* (Figures 3 and 4, Table I). Protein levels in the podia and pyloric caeca were 373 mg/g dry weight and 285 mg/g dry weight respectively at the beginning of the experiment. There were little changes in these levels in tissues of either the control or hyposmotically stressed animals.

Ammonia excretion

Ammonia excretion by hyposmotically stressed animals was higher than by control animals throughout the 52 hours of measurement (Fig. 5). The peak of

TABLE I

Changes with time in the expected and observed levels of ninhydrin positive substances (mg NPS/g tissue water) in the podia and pyloric caeca of hyposmotically stressed Luidia clathrata. *Expected values were calculated on the basis of the NPS levels in control tissues and on the increase in hydration in hyposmotic tissues. Observed values are means ± s.d. ($n = 5$)*

Sampling time	1 Day	2 Days	4 Days	6 Days	8 Days	10Days
Podia						
Expected NPS	10.18	9.38	9.89	12.92	13.28	13.34
Observed NPS	6.82	6.96	5.89	3.53	5.57	6.92
s.d.	1.40	2.17	1.35	0.79	1.07	0.75
Pyloric caeca						
Expected NPS	10.66	8.21	10.43	9.87	10.10	9.71
Observed NPS	5.07	7.52	6.12	5.13	4.46	6.22
s.d.	0.58	0.95	1.15	3.38	1.16	1.51

ammonia excretion occurred 26 hours after the initiation of stress. At this time, the mean value of ammonia excretion by hyposmotically stressed animals was almost double that by control animals. The excretion rates declined after this time in the hyposmotically stressed animals and approached the rate of the control animals at 52 hours. There was considerable variation in excretion rates of control and hyposmotically stressed animals. As a consequence, the mean excretion rates of the two groups were not significantly different at the 95% level except for the 26 hour sampling time.

DISCUSSION

An increase in the whole animal volume of hyposmotically stressed specimens of *Luidia clathrata* can be related in part to an increase in tissue hydration. The extent of the increase, however, indicates that most of the volume increase results from the dilution of the coelomic fluid. The subsequent decline in volume of

hyposmotically stressed animals with time suggests a degree of volume regulation similar to that found by Virkar (1966) for the sipunculid *Golfingia* and by Pierce (1971b) for two species of the bivalve *Modiolus*. This observation of volume regulation by specimens of *Luidia* contrasts to its reported lack in the echinoid *Strongylocentrotus purpuratus* (Giese and Farmanfarmaian, 1963) and in *Asterias rubens* from a relatively stenohaline population (Binyon, 1961). Binyon (1972a) suggests that earlier reports that *A. rubens* showed volume regulation to be due to mechanical damage induced in handling. There was no observation of such damage to the integument of specimens of *Luidia* in this experiment. The asteroid, *Odontaster validus*, has the ability to retain its original weight in 50% sea water (Pearse, 1967). Thus, there may be at least a limited capacity for regulation of

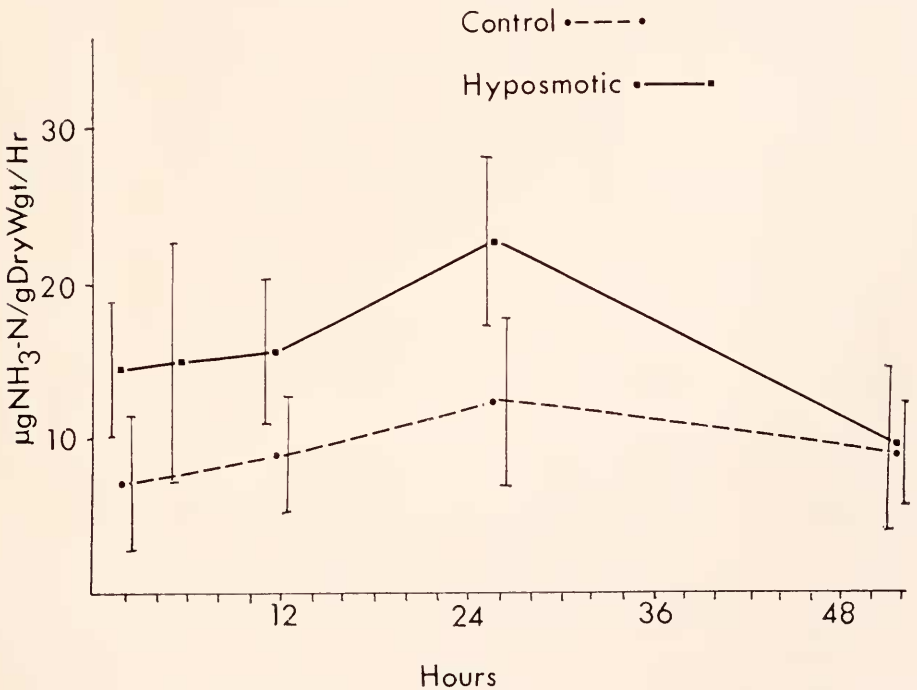

FIGURE 5. Changes with time in the rates of ammonia excretion of *Luidia clathrata* maintained at control (ambient) salinity (27‰) and at hyposmotic salinity (16‰). Points represent means (n = 5); vertical lines represent ± 1 s.d.

the coelomic fluid in some asteroids. Binyon (1972a) suggests that there may be an optimal coelomic volume to body volume ratio which tends to be maintained.

As indicated by the effect on the activity coefficient, hyposmotic stress on specimens of *Luidia* results in a temporary loss of locomotory capacity. The ability to recover quickly locomotor activity after dilution of its medium obviously has important ecological significance for this species in bays where such effects can occur suddenly. The ability to recover locomotor activity with hyposmotic stress has been reported for *A. rubens* from the coast of France (Jeuniaux *et al.*, 1962).

Binyon (1972b) reported that a population of *A. rubens* from the coast of Great Britain had a much less capacity to recover locomotor ability. Recovery of activity in hyposomotic conditions has also been found for the echinoid, *S. droebachiensis* (Lange, 1964).

The increase in tissue hydration levels found here for specimens of *Luidia* is similar to that reported for other echinoderms. Kowalski (1955) found that the per cent hydration of ovarian tissue of *A. rubens* was lower in animals from the North Sea (30–32‰) than from the Baltic Sea (15–17‰) with mean per cent hydration values of 80.5 and 84.0 respectively. It has been shown experimentally that the per cent hydration increases with hyposmotic stress in the pyloric caeca (Jeuniaux *et al.*, 1962) and podia (Binyon, 1972b) of *A. rubens*. In both cases, it was concluded that the smaller than expected increase in tissue hydration was related to isosmotic intracellular regulation. The per cent hydration of the gut of the echinoid, *S. droebachiensis*, is increased with hyposmotic stress (Lange, 1964). Lange (1970) stated that water molecules must replace free amino acids lost during cell volume regulation. Thus, the per cent hydration levels of tissues of hyposmotically stressed animals should be somewhat higher than levels of control animals if there is complete adjustment. The amount of decrease in the level of hydration of podia of hyposmotically stressed specimens of *Luidia* is not in accord with this generalization. Variability resulting from the method of absorbing the ambulacral fluid may be responsible for the discrepancy.

The decrease in levels of NPS within 24 hours after the beginning of hyposmotic stress indicates a removal of these molecules from the cells of this species. Virkar and Webb (1970) also found that the absolute response of the free amino acid pool of the hyposmotically stressed clam, *Mya*, is immediate. As the decrease in NPS is far greater than that predicted on the basis of changes in per cent hydration of the tissues of *Luidia clathrata*, the decrease in NPS represents an actual loss and not a dilution of the free amino acid pool. This decrease in the pool has been shown for several bivalve species (Virkar and Webb, 1970; Pierce, 1971a).

A decrease in the levels of tissue NPS with hyposmotic stress has also been reported for the pyloric caeca (Jeuniaux *et al.*, 1962) and the podia (Binyon, 1972b) of *A. rubens* and the gut of the echinoid, *S. droebachiensis* (Lange, 1964). These workers concluded that amino acids were the prime effectors of the isosmotic intracellular regulation in these echinoderms. The change in the NPS levels in the pyloric caeca of specimens of *Luidia* were in proportion to the dilution of the medium, but the changes in the podia were greater than would be expected for compensation. Lange (1964) found that the intestinal levels of NPS in *S. droebachiensis* were a linear function of sea water concentration. Virkar (1966) found a non-linear response of NPS to sea water concentration in the sipunculid, *Golfingia*. Analysis of the levels of NPS in the podia of specimens of *Luidia* at more salinity levels is necessary to ascertain if a non-linear response is occurring here also.

A possible route for the loss of free amino acids in this species would be the deamination and catabolism of the carbon skeletons. The increase in the rate of ammonia excretion in hyposmotically stressed specimens of *Luidia* suggests that this may be occurring. An increase in ammonia excretion associated with hyposmotic stress has been reported in the holothurian, *Eupentacta quinquesemita* and the

echinoid, *S. droebachiensis* (Emerson, 1969). The increase in ammonia excretion in *Luidia clathrata* is immediate, as has been found also in the polychaete, *Nereis virens*, and the shore crab, *Carcinus maenas* (Haas, Haberfield and Hammen, 1972).

Schoffeniels and Gilles (1972) suggest that the level of organic effectors is regulated by a control of the synthesis-degradation equilibrium. An activation of enzymes involved in degradation would result in an increase in ammonia excretion. It is possible that hyposmotic stress may result in the activation of specific enzymes involved in amino acid catabolism. An appropriate class of enzymes are the L-amino acid oxidases. Activation of these enzymes of oxidative deamination would result in a decrease in the free amino acid pool, an increase in ammonia excretion, and possibly the often observed phenomenon of an increase in oxygen consumption. Preliminary measurements indicate that there are negligible amino acid deaminases in the podia and pyloric caeca of specimens of *Luidia* (F. E. Friedl, unpublished data). This does not exclude the possibility of highly specific deaminating enzymes. Simpson, Allen and Awapara (1959) reported high concentrations of glycine and taurine in this species. Osmotic stress may result in the activation of enzymes involved in the relatively complex degradative pathways of these two compounds. Since an increase in ammonia excretion with hyposmotic stress occurs in a number of phyla, there may be a uniformity in the molecular events involved in this process.

We conclude that *Luidia clathrata* has two mechanisms which allow it to be euryhaline and able to function in an environment of fluctuating low salinities. These are the ability to partially regulate the volume of its coelomic fluid and to carry on intracellular isosmotic regulation. *Luidia clathrata* apparently is the only member of its genus to be euryhaline. Another member, *L. sarsi,* is stenohaline. Ursin (1960) reported that it is never found at salinities below 30‰. Lower salinities may diminish or even exterminate populations of *L. sarsi* (Fenchel, 1965). The occurrence of euryhalinity in *L. clathrata* is of significance in that it indicates that even an evolutionary primitive group of the class Asterozoa has the capacity to develop this ability.

We thank Dr. F. E. Friedl of the University of South Florida for performing the enzyme assays and for stimulating ideas concerning the molecular basis of the phenomena. We also thank K. Ellington and E. Johnston for their technical assistance.

Summary

(1) The asteroid *Luidia clathrata* becomes inactive when transferred from 27‰ sea water to 16‰, but recovers its activity within 48 hours.

(2) The volume of intact animals increases with the initiation of the hyposmotic stress, but returns to somewhat above the original level within several days. This suggests regulation of the volume of the coelomic fluid.

(3) The changes in the per cent hydration and level of ninhydrin positive substances in the podia and pyloric caeca of hyposmotically stressed animals indicate that intracellular isosmotic regulation occurs.

(4) The increase in the rate of ammonia excretion of hyposmotically stressed animals suggests that the decrease in tissue NPS levels results from their catabolism.

(5) *Luidia clathrata* is a euryhaline echinoderm, responding to reduced salinities by coelomic fluid volume regulation and isosmotic intracellular regulation.

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